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Multilocus Inherited Neoplasia Alleles Syndrome (MINAS): Case Series and Literature

Review

James Whitworth MRCP¹, Anne-Bine Skytte MD², Lone Sunde MD², Derek H Lim MRCP³, Mark J. Arends PhD FRCPATH⁴, Lisa Happerfield MSc⁵, Ian M Frayling PhD FRCPATH⁶, Rick van Minkelen PhD⁷, Emma R Woodward PhD MRCP⁷, Marc D Tischkowitz PhD MRCP¹, Eamonn R Maher FRCP FMedSci¹.

¹Department of Medical Genetics, University of Cambridge and Cambridge NIHR Biomedical Research Centre, Cambridge CB2 0QQ, UK.

²Department of Clinical Genetics, Aarhus University Hospital, Denmark.

³West Midlands Regional Genetics Service, Birmingham Women's Hospital NHS Trust, Birmingham, UK.

⁴ Division of Pathology, University of Edinburgh and Institute for Genetics & Molecular Medicine, Western General Hospital, Edinburgh, UK.

⁵Department of Pathology, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ

⁶Institute of Cancer and Genetics, Cardiff University School of Medicine, UK

⁷Department of Clinical Genetics, Erasmus Medical Center, Rotterdam, The Netherlands

⁸Department of Genomic Medicine, St. Mary's Hospital, Central Manchester University Hospitals NHS Foundation Trust, Manchester Academic Health Science Centre, Manchester, UK.

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Correspondence to:

Professor Eamonn Maher, Department of Medical Genetics, University of Cambridge, Cambridge Biomedical Campus, Addenbrooke's Treatment Centre, Cambridge CB2 2QQ, UK

Telephone: (+44) 01223 746714. Email: erm1000@medschl.cam.ac.uk

Abstract

Mendelian causes of inherited cancer susceptibility are mostly rare and characterized by variable expression and incomplete penetrance. Phenotypic variability may result from a range of causes including locus heterogeneity, allelic heterogeneity, genetic and environmental modifier effects or chance. Another potential cause is the presence of two or more inherited cancer predisposition alleles in the same individual. Though the frequency of such occurrences might be predicted to be low, such cases have probably been under ascertained because standard clinical practice has been to test candidate inherited cancer genes sequentially until a pathogenic mutation is detected. However, recent advances in next generation sequencing technologies now provide the opportunity to perform simultaneous parallel testing of large numbers of inherited cancer genes. Here we provide examples of patients who harbor pathogenic mutations in multiple inherited cancer genes and review previously published examples to illustrate the complex genotype-phenotype relationships in these cases. We suggest that clinicians should proactively consider the likelihood of this phenomenon (referred to here as Multilocus Inherited Neoplasia Alleles Syndrome (MINAS)) in patients with unusual inherited cancer syndrome phenotypes. To facilitate the clinical management of novel cases of MINAS we have established a database to collect information on what is likely to be an increasingly recognized cohort of such individuals.

Introduction

In clinical practice the maxim of Occum's razor is often adopted¹ such that, whenever possible, a single diagnosis is favored over multiple diagnoses. Rare diseases have a frequency of less than 1 in 2000² and statistically the chances of an individual being affected by two or more rare diseases would appear to be remote. However, with more than 6,000 rare diseases and up to 6-8% of the European population estimated to suffer from a rare disease at some time in their lifetime², there is potential for two or more rare disorders to occur by chance. This scenario has been reported in various constitutional disorders with both distinct and overlapping phenotypes, including familial neoplasia and/or patients with multiple primary tumors. If Occum's razor is applied then the detection of a mutation in a specific inherited cancer gene might lead the clinician to attribute any tumors that are not typical features of the relevant inherited cancer syndrome to examples of variable phenotypic expression or coincidence. In such circumstances the patient may receive suboptimal management and the estimated cancer risks to relatives could be erroneous. In addition, studies of patients harboring multiple mutations in different familial cancer syndrome genes could provide insights into how the function of the relevant gene products may be related e.g. if a particular combination resulted in a more pronounced or novel phenotype (analogous to the differences in phenotype between patients with monoallelic and biallelic mismatch repair (MMR) gene mutations³). The best known examples of patients with multiple inherited cancer gene mutations are reports of patients with mutations in *BRCA1* and *BRCA2*⁴⁻²². Interestingly, the phenotype in these patients has generally not been shown to be more severe than when a single mutation is present.

Here we report five new cases with multiple pathogenic germline mutations in rare inherited cancer syndrome genes. Three involve the combination of mutations in *FLCN* with *NF1*, *TP53* and *MSH2* respectively, one in *MLH1* and *XPA* and the fifth in *NF1* and *BRCA2*. In addition, we reviewed the published literature to identify other cases and provide a summary of the published experience to date. We suggest that this phenomenon will be increasingly recognized and careful descriptions of such cases will inform the management of similar patients.

Case Reports

Case 1:

A 39 year old man presented with testicular seminoma and a routine abdominal scan four years later revealed a pheochromocytoma. Following his seminoma diagnosis he also developed a pneumothorax and went on to have six further occurrences. At age 55 years he complained of abdominal/ back pain and a CT scan revealed bilateral renal masses that were demonstrated to be renal cell carcinomas (RCC) following removal. Reinvestigation following further episodes of abdominal pain identified two gastrointestinal stromal tumors (GIST). At age 56 years, a CT lung scan (to investigate a pneumothorax) revealed a malignant peripheral nerve sheath tumour (MPNT). Skin examination revealed multiple skin neurofibromas, two café au lait patches and axillary freckling but no fibrofolliculomas. A clinical diagnosis of neurofibromatosis type 1 (NF1) was made and though this was considered to be the cause of his MPNT and possibly pheochromocytoma and GIST, the history of renal cancers and recurrent pneumothorax were considered unrelated.

Next generation sequencing (NGS) of 94 inherited cancer genes was performed using the Illumina TruSight cancer panel²³. A previously reported splice site mutation in *FLCN* (c.1062+2T>G)^{24,25} and a nonsense mutation in *NF1* (c.1381C>T p.(Arg461*)) were detected and confirmed by Sanger sequencing. *FLCN* mutations cause Birt-Hogg-Dube syndrome (BHD), a rare condition where affected individuals are predisposed to RCC, pulmonary cysts and pneumothoraces and fibrofolliculomas. A first degree relative had also been diagnosed with bilateral chromophobe RCCs at age 45 years and was found to have facial fibrofolliculomas though did not have genetic testing. Presence of the *FLCN* mutation was demonstrated in a further first and second degree relative but both were asymptomatic with normal renal scans. It was also identified in a second degree relative with numerous fibrofolliculomas and a history of recurrent pneumothorax (x2). An obligate carrier in the family had pancreatic adenocarcinoma but was not known to have features of BHD syndrome during life, although autopsy revealed bilateral renal oncocytomas. There was no known family history of NF1.

NF1 has a population frequency of 23/100.000²⁶ and might be expected to exist in combination with another inherited cancer syndrome relatively rarely, though phenotypic variability and use of clinical diagnostic criteria (rather than genetic testing) may underestimate this. It is associated with predisposition to a variety of neoplasms including pheochromocytoma, GIST, carcinoid tumour, cutaneous/plexiform neurofibromas and MPNT. Thus in this case associated with two pathogenic inherited cancer syndrome gene mutations, the occurrence of the MPNT, phaeochromocytoma, GIST and RCC can be explained but testicular seminoma has not been associated with mutations in either gene^{27,28}. This suggests that the seminoma might be a consequence of the combination of *FLCN* and *NF1* mutations (seminoma has been linked to aberrations in the c-kit, RAS/MAPK and PI3K/Akt pathways²⁹ and the *NF1* and *FLCN* gene products regulate RAS/MAPK and mTOR/PI3K/Akt signaling respectively^{29,30}) or be coincidental, testicular the most common male solid tumour in the 15-34 age group³¹.

Case 2:

A 32 year old man presented with dysphagia. There was a previous history of ulcerative colitis for which he had undergone a pan-proctocolectomy at age 27 years and pathological examination of the colectomy specimen had revealed an incidental rectal adenocarcinoma. Endoscopy revealed a gastroesophageal junction adenocarcinoma and staging imaging demonstrated a 6cm left kidney tumor. Biopsy of the latter suggested a primary renal neoplasm, prompting nephrectomy. Histology of the resected kidney confirmed a chromophobe RCC. Examination of the skin showed facial fibrofolliculomas. There was no history of cancer in first degree relatives but tumours in second degree relatives included oesophageal squamous cell carcinoma at age 54 years , a brain tumor of uncertain histology at age 50 and an oropharyngeal carcinoma at 49 years.

Genetic investigations revealed two pathogenic mutations in *FLCN* (c.715C>T p.(Arg239Cys))³² and *TP53* (c.526T>C p.(Cys176Arg)). The latter has been reported as a somatic mutation on multiple occasions^{33,34}, including in colorectal adenocarcinoma³³ but not previously in germline samples³⁴. It is rare and does not appear in the ExAC dataset³⁵. *In silico* tools predict a damaging or function altering effect³⁶⁻³⁸. No other family members were available for genetic testing.

Kidney tumors, typically with a hybrid chromophobe/oncocytic RCC histopathology, are a major feature of BHD syndrome. RCC has been reported in *TP53* mutation carriers though no firm association been made³⁹. The relationship between colorectal cancer and BHD syndrome is controversial^{24,40} but an increased risk of colorectal cancer has been reported with ulcerative colitis (though typically in those with disease for >10 years⁴¹) and also in *TP53* mutation carriers. To our knowledge, esophageal cancers have not been reported in *FLCN* mutation carriers but have occurred in Li-Fraumeni syndrome (LFS) families, though again the association with this condition is not clear^{39,42}. We note that the median age at diagnosis of renal tumors in *FLCN* mutation carriers (48 years)²⁵ is older than the age at onset of these tumors in this case.

Case 3:

A 53 year old woman presented with a rectal adenocarcinoma and had a history of spontaneous pneumothorax at age 46 years. . Immunohistochemistry performed on the proband's rectal tumour showed no abnormality but a relative's (who also had multiple pneumothoraces) colon cancer demonstrated loss of staining of MSH2 and MSH6 proteins. Germline genetic testing in the proband did not detect a pathogenic mismatch repair gene mutation but a pathogenic *FLCN* mutation (c.1285delC p.(His429Thrfs*39)) was identified. Three siblings had phenotypic similarities to the proband. A sister developed a pneumothorax at age 37 and had facial fibrofolliculomas. She developed endometrial cancer at 52 years. Genetic testing demonstrated the familial *FLCN* mutation and a *MSH2* truncating mutation (c.892C>T p.(Gln298*)). The twin sister of this individual had pneumothoraces, RCC and colorectal polyps. She also carried both mutations, as did a brother with facial fibrofolliculomas.

Colorectal and endometrial cancers are characteristic of Lynch syndrome caused by *MSH2* mutations and the ages of diagnosis seen in this family are typical⁴³. However, the proband did not carry the pathogenic *MSH2* mutation detected in her siblings and may represent a phenocopy. Also, a role of the *FLCN* mutation in the development of colorectal tumours in the family cannot be excluded^{24,40}. Fibrofolliculomas, RCC and pneumothoraces are not associated with Lynch

syndrome⁴⁴.

Case 4:

A male proband presented with a mucinous caecal cancer at age 65 years and a metachronous sigmoid colon cancer in his remaining large bowel at 67 years. One first degree relative had developed colon cancer at age 42, but there was no other family history of Lynch syndrome-related tumours. His parents were not knowingly consanguineous, but were both from the same small community in India. The proband had been clinically diagnosed in early childhood with xeroderma pigmentosum (XP). At least one other first degree relative was known to have a similar pattern of skin tumours, but that individual had no internal malignancies. Neither parent had any reported skin abnormalities. On examination his sun-exposed skin showed considerable signs of ultraviolet damage (e.g. severe freckling and loss of pigment) but no other features of XP such as neurological or intellectual deficits. His skin tumours over the previous 20 years had included a squamous carcinoma in an actinic keratosis, several seborrheic keratoses, two keratoacanthomata/squamous carcinomas, junctional nevi, a squamous carcinoma and two lentigo malignae (pre-malignant melanoma). Immunohistochemistry demonstrated loss of MLH1 and PMS2 expression in both colon cancers. Constitutional genetic testing revealed *MLH1* c.306G>T p.(Glu102Asp) (classified as likely pathogenic⁴⁵). Fibroblasts from a skin biopsy were tested for XP, which showed reduced levels of nucleotide excision repair. He therefore did not have mild XP variant (XP-V) as might be expected, but rather had mild variant XP-A, consistent with survival into his 60s. Constitutional genetics analysis revealed a homozygous *XPA* intron 4 splice mutation (c.555+8A>G). Molecular analysis of his various tumours is summarised in Table 1.

The prevalence of microsatellite instability (MSI) in skin tumours in XP is unknown. A contribution of the *MLH1* mutation to the dermatological phenotype may be suggested by its presence in some of the skin tumours but the presence of normal MLH1 and PMS2 expression goes against this. Skin tumours are associated with Lynch syndrome but these are characteristically sebaceous in origin, which were not observed in this case.

Case 5:

A female patient with NF1, having one café au lait patch, numerous cutaneous neurofibromas, possible Lisch nodules and a MPNT, was diagnosed with a ductal breast carcinoma at age 48 years and subsequently went on to develop a cutaneous melanoma at age 57 years.

Constitutional genetic testing revealed both *NF1* c.6792C>G p.(Tyr2264*) and *BRCA2* c.5213_5216del p.(Thr1738Ilefs*2)⁴⁶. Mutations in both genes can be associated with breast cancer⁴⁷ but the risk is much higher for *BRCA2*. The breast cancer could be consistent with either syndrome and no tumor analysis was reported that could help determine which gene was more significant in its initiation.

Having identified five cases harbouring multilocus inherited neoplasia gene mutations we proceeded to review the published literature to determine the nature and frequency of similar cases in a systematic fashion. We propose the term “Multilocus Inherited Neoplasia Alleles Syndrome” (MINAS) to describe this phenomenon.

Literature Survey of Multilocus Inherited Neoplasia Alleles Syndrome

Identification of cases

To review published cases with MINAS we undertook a systematic review of the published literature (see Supplementary Methodology section) based on a list of inherited cancer genes (n=94) (Supplementary Table 1).

Clinical Aspects

82 cases involving 17 inherited cancer genes were identified^{4–7,20,48–69} (see Supplementary Table 2). The combination of co-existing mutations in *BRCA1/BRCA2*, *BRCA2/TP53*, *BRCA1/MLH1* and *APC/MLH1* were the only combinations that occurred in more than one family. This may reflect ascertainment bias (certain genes are commonly screened for simultaneously), common founder mutations present in specific populations and hereditary breast cancer, followed by colorectal cancer, being the most common indication for cancer genetic assessment⁷⁰. Indeed, 13 patients had a combination of two of the three Ashkenazi founder *BRCA1* and *BRCA2* mutations.

An interesting aspect of patients with MINAS is whether mutations in particular combinations of genes are associated with a more (or less) severe phenotype (e.g. earlier onset of cancer or cancer types not usually seen in individuals with a single mutation). The wide variety of combinations of individual germline mutations means that, with the exception of *BRCA1/BRCA2*, mutation combinations the information on phenotypic effects is limited.

Leegte et al⁸ described 12 cases of combined *BRCA1/BRCA2* mutation cases and suggested that there was no evidence of increased severity whereas Heidemann et al⁷ reported eight cases and suggested that a more severe phenotype was observed in two. Other combinations of inherited breast cancer genes have been described. For example, a combination of mutations in *BRCA1* and *PALB2* was described in a patient with multifocal breast cancer⁶⁷ (Case 25, Supplementary Table 2). Uterine leiomyomas and a meningioma were also diagnosed but it is impossible to know whether these were related to a specific mutations or were coincidental.

Two reports of germline *BRCA2* and *TP53* mutations were identified^{53,54}. In a mouse model where the homologues of both of these genes are conditionally knocked out in epithelial tissues (to avoid embryonic lethality), a greater incidence and earlier onset of mammary and skin carcinomas was observed in comparison to mice where only *Trp53* or *Brca2* was conditionally knocked out (with conditional knockout/wild type heterozygosity in the other gene), suggesting a synergistic effect in these tissues⁷². Though the mouse model is not directly comparable to the human status, more than two cancers had occurred in both cases of *BRCA2/TP53* MINAS, though one tumor was within the radiotherapy field and the ages of diagnosis in these cases are not atypical for mutations in either gene^{39,73}.

The second most frequently reported examples of specific MINAS were combinations of genes predisposing to inherited colorectal cancers^{62–66} (cases 20–24, Supplementary Table 2).

Interestingly, severe phenotypes were noted in two patients with *APC/MLH1* mutation combinations with jejunal cancer seen in one case⁶² and accelerated polyp progression in the other⁶⁵.

The phenotypic consequences of MINAS may be easier to interpret when the two genes involved are associated with dissimilar and narrow phenotypes. Thus, there are various reports of a *BRCA1/BRCA2* mutation in combination with a mismatch repair gene mutation (see cases 1-4, Supplementary Table 2). In general these have not demonstrated clear evidence of a synergistic effect of these types of mutations on the severity of the phenotype although one reported case with *BRCA1* and *MLH1* mutations had a severe phenotype involving early onset bilateral breast cancer, and endometrial, ovarian and clear cell renal cancers diagnosed at age 39 years. Both breast tumors showed loss of the wild-type *BRCA1* allele but also showed absent staining of *MLH1* on immunohistochemistry and loss of the wild-type *MLH1* allele. This suggests that both germline mutations were significant in breast tumorigenesis in this patient. The high number of tumors and the development of early onset RCC (not usually associated with *BRCA1* or *MLH1* mutations) suggests a possible synergistic effect⁴⁹.

Reports of other MINAS cases with specific gene combinations are rare. For example *PTEN* mutations, which affect the PI3K/Akt signaling pathway^{74,75} are reported in combination with mutations in *TP53*⁶⁹, *APC*⁶⁰ and *SDHC*⁶¹ with tumors characteristic of each mutation being observed in all three cases. A number of the tumors in the *PTEN/TP53* case were not typical of a mutation in either gene and early onset of colonic polyps and paraganglioma were noted in the other patients. *PTEN* normally acts via Akt to down regulate MDM2 (and therefore increase p53 levels) in addition to its other roles^{74,75} so this interaction may lead to a more severe phenotype. A further case of *BRCA1* and *NF1* mutations in a patient with cutaneous features of NF1 and early onset (age 35) breast cancer has also been described⁶⁹. Of note is the fact that *NF1* and *BRCA1* are both located on the long arm of chromosome 17. The presence of early onset breast cancer and NF1 in the patient's mother along with both mutations being found in the proband may suggest that the two altered genes were *in cis*. Such information has significant implications for genetic counselling of families where multiple mutations are identified though interestingly, the probands brother, who also had NF1, did not carry the *BRCA1* mutation suggesting a recombination event in the mother.

A case of MINAS involving a *FLCN* and *APC* mutations has been reported⁵⁶. Typical colonic polyps and a colorectal cancer at age 28 occurred, as well as recurrent pneumothoraces and facial papules. The features are consistent with an independent mechanism, though the authors suggested that the *FLCN* mutation might have enhanced the tumorigenic process given the observation that somatic *FLCN* mutations frequently occur in (microsatellite unstable) colorectal cancers⁴⁰.

Molecular Genetics Aspects

In theory, insights into the role of individual inherited cancer gene mutations in the pathogenesis of tumor types that are rarely associated with either of the relevant genes (or tumor types associated with both genes) might be derived from loss of heterozygosity (LOH) studies (assuming the relevant inherited cancer genes are tumor suppressor genes (TSGs)). LOH analysis, however, can be uninformative if the somatic mutation ("second hit") is a point mutation or promoter methylation of the wild-type allele (i.e. no LOH)⁵. For example, LOH analysis of three primary breast cancers from a woman with *BRCA1/BRCA2* MINAS demonstrated LOH at *BRCA1* in one tumor and at *BRCA2* in the other two- suggesting that there was no direct interaction between the two loci in the tumors. However, in another case report of *BRCA1/BRCA2* MINAS, LOH at both loci was demonstrated in an ovarian cancer from the same patient¹⁹.

Future Perspectives

There are inherent ascertainment biases influencing which MINAS cases are present in the literature including more frequent analysis of combinations of particular genes, the range of phenotypes referred for testing and the restriction of analyzed genes to only those most strongly suggested by the tumor history. Availability, or lack thereof, of analysis of certain genes in some centers may also be a factor and is likely to have led to recognition of three *FLCN* MINAS cases at our center where this gene is tested frequently. Clinical features as the skin manifestations of BHD syndrome and NF1 can indicate the need for analysis of specific genes but increasing use of cancer gene panels or whole exome/genome sequencing provides the opportunity for a more comprehensive genetic testing strategy and is likely to result in increased recognition of cases of

MINAS. Increasing detection will inevitably lead to increased demand for accurate information on whether particular combinations of mutations are likely to result in a particularly severe phenotype (i.e. a synergistic interaction) or whether the resulting phenotype is typical of each mutation having an independent effect. Review of previous reports of MINAS reveals that although a more severe phenotype seems likely in some cases, this cannot be concluded in the majority. *In utero* death from more severe manifestations of mutation combinations may account for some milder phenotypes but where survival occurs, MINAS may be more likely suspected in severe cases or those with an atypical phenotype. We suggest that as further cases are uncovered by routine multigene testing strategies, those cases with a less severe phenotype will be recognized more easily. However, in certain circumstances it may be prudent to expect that a particular combination of mutations might result in a more severe phenotype. Thus, if an individual has mutations in TSGs that map to the same chromosome region, loss of a chromosome (or part of) harboring the wild type alleles will result in a tumor homozygous null for both TSGs (this may have occurred in Case 2 as *FLCN* and *TP53* map to 17p11.2 and 17p13.1 respectively). Also if there is a direct relationship between the mechanisms of tumorigenesis of the two mutations (e.g. *APC* and mismatch repair gene mutations) a more severe phenotype may occur. In addition, two gain-of-function mutations in proto-oncogenes might predict a more severe phenotype (though we have not found reports of such cases) because, in contrast to TSGs, an additional event (somatic inactivation of a wild-type allele) is not required to initiate tumorigenesis. As mutation-dependent targeted therapies for the treatment of cancers become a more common option in oncology, the recognition of MINAS and application of tumor analysis to define the most likely driver mutation will become more important.

The optimum resource with which to discern the effects of individually rare mutation combinations and improve future management of patients with MINAS is a reference database containing clinical, genetic and tumor information. Such information could guide the clinician as to what the effect of each combination of mutations might be. To facilitate sharing of such information, cases can be uploaded to the Leiden Open Variant Database and identified by “MINAS” phenotype (<http://databases.lovd.nl/shared/diseases/04296>). We hope that other oncology and genetics health

care professionals and researchers will contribute their cases in order to increase knowledge of this emerging phenomenon.

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Table 1: Molecular analysis of tumors from Case 4

Tumour	MLH1 IHC	PMS2 IHC	MSI assessment
Mucinous caecal adenocarcinoma	Loss	Loss	High
Sigmoid colon adenocarcinoma	Loss	Loss	High
Squamous carcinoma (#1)	Present	Present	Stable
Squamous carcinoma (#2)	Present	Present	Stable
Lentigo maligna	Present	Present	High
Actinic keratosis	Present	Present	High
Squamous carcinoma in actinic keratosis	Present	Present	High

Key:

MSI - Microsatellite instability. IHC - Immunohistochemistry